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Serial batch extraction of zein from milled maize☆

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Abstract

Prolamine-rich, water insoluble proteins (zeins) can be extracted from milled maize by vigorous mixing in heated ethanol solutions. Whenever solvent extraction is used process cost considerations require that all the solvent be recovered. Because of the low zein content of maize rinsing extract liquid from the extracted maize particles must be done in a way that minimizes dilution. The solid mass fraction of milled grain slurry that can be pumped is 0.25 or less. Because the mass fraction of zein in maize is only approximately 0.05, the zein mass fraction of a batch extract will be less than 0.015. To increase the zein concentration, a batch extract (liquid and fines) can be repeatedly separated from the extracted solid particles and used to extract fresh milled grain. A series of batch extractions with extract reuse can approach the performance of a continuous counter-current solids/liquid extraction, which would be preferable at a commercial scale. Extract reuse is constrained by losses of liquid with the extracted grain and by reductions of the extracting capacity of the extract due to the increasing solute or fines content. By examining the extract composition and yield of a series of batches, it is possible to estimate the zein concentration that could be achieved in a continuous, countercurrent process and to examine effects of higher zein concentrations on extraction that would be inaccessible with a single batch. The centrifugate concentrations for a series of maize extractions in which the extracted maize and extract solution were cooled prior to centrifugation were analyzed. The data were fit with a model based on a maximum zein concentration in the extract. The fit indicates that the protein content of the liquid centrifugate will not exceed 2% for any series of similar batch extractions, by using centrifugation to separate the maize from the extraction slurry after cooling it to ambient temperature. This contrasts with concentrations of zein of 10% or more achieved by extracting corn gluten using similar conditions. Although the concentration of zein in gluten is higher we believe the concentration difference is mainly due to chemical changes to the zein that take place in the gluten production and the methods used to extract zein from gluten. Published by Elsevier Science B.V.

Keywords: Extraction; Precipitation; Centrifugation; Zein

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1. Introduction

Prolamine-rich, water insoluble proteins (zein) can be extracted from milled maize by vigorous mixing in heated ethanol solutions. Other grasses contain protein similar to zein, kafirins from sorghum and coixins from coix, for example (Garratt et al., 1993). Although a relatively small amount of zein is produced from corn gluten, no other zein type proteins are commercially extracted from grain. Recent studies suggest that extraction of zein from maize could be commercially feasible as part of a starch to ethanol conversion process (Dickey et al., 1999; Shukla et al., 2000).

Zein will precipitate from a filtered or centrifuged extract after dilution to 40 wt.% ethanol. Although concentrated solutions can be prepared by re-dissolving the precipitated zein, its direct preparation is preferred to avoid possible irreversible changes to the protein due to precipitation, until final use. Thus, it is desirable to extract the maize using a procedure that results in a high zein concentration. This should also entail using as little ethanol as possible to minimize extraction cost, since ethanol re-concentration by distillation for reuse is the greatest part of the overall process cost.

The solid mass fraction of a milled grain slurry that can be pumped is 0.25 or less. The solid fraction, and the low zein fraction of grain, approximately 0.05 (Landry, 1997), limits the zein mass fraction of a single batch extract to less than 0.015. This concentration is too low for convenient storage, transportation or use.

To increase its zein concentration, extract can be used to extract more milled grain. Repeated extract use to extract new batches of grain will ultimately be limited by losses of liquid with the extracted grain and also, possibly, by reduction of the extracting capacity of the liquid due to the interference of increased solute (zein and lipid) as the saturation limit is approached. It has recently been shown that zein is associated with free fatty acid in protein bodies and alcohol extracts (Forato et al., 2000). Liquid losses can be easily measured and depend upon the method used to separate the extract from the grain slurry.

The experiments described herein used a decanter centrifuge to produce a 55% solids stream and an extract stream containing liquid and fine solids. The composition and yield of the extract liquid was determined after each batch of a series of batches, with extract reuse. This information was used to estimate zein concentration for a continuous, countercurrent process.

2. Materials and methods

2.1. Maize preparation

The yellow dent maize meal from a commercial feed mill (Davis Feed Mill, Perkasie, PA) was used. The shelled maize was cracked with a roller mill and the pericarp removed by aspiration. The cracked maize was passed through a counter-rotating, ribbed disc mill and reduced to a median size of 0.35 mm, as determined by sieve separation of 23 kg of meal. Although it was not possible to control the maize hybrid from the mill, all milled corn used for a series of extractions was purchased at the same time and stored in a cold chamber at -20 °C before extraction.

2.2. Analysis

The protein content ($N \times 6.5$) was determined by the micro-Kjeldahl method (AACC, 1995; AOAC, 1995). Oil content was determined by packing a pipette plugged with glass wool with 100–300 mg of sample, previously dried at 110 °C overnight. The column was eluted with 5 ml of hexane followed by 5 ml of chloroform. Elutes were collected in tared vials and subsequently evaporated to constant weight with nitrogen and the weights of hexane and chloroform extracts determined. Solid content was determined by weighing samples before and after over-night heating to 100 °C. The ethanol contents of the extract solutions and diluted centrifugates were calculated from liquid density and temperature measurements. Solution densities were measured with calibrated hydrometers (Ever Ready Thermometer Co., Inc., West Patterson, NJ). The ethanol concentration calculations were confirmed by

HPLC measurements of some samples. To accomplish the HPLC measurement the solutions were diluted 1:25 with water to precipitate the zein. The mixture was then filtered through a 0.45-μm membrane and injected into a HPLC equipped with a Bio-Rad Fast Acid Analysis column (100 × 7.8 mm) maintained at 70 °C. The column was eluted with 0.001 M sulfuric acid at 0.7 ml/min and the amount of ethanol was detected as the area under a peak using a refractive index detector. The concentrations calculated from the densities agreed with HPLC measurements within 1.5%.

2.3. Extraction and maize separation

Four series of extractions, i.e. sets of batches in which extract from a preceding batch was used to extract milled maize, were carried out, the first three at 50 °C. A detailed schematic that shows the extraction process is presented in Fig. 1. In the first, four 68-kg batches of milled maize were extracted, each for 60 min. In the second, seven 90-kg batches of the milled maize were extracted for 60–90 min. In the third, three 90-kg batches of the milled maize were extracted, the first for 60 min, the last two for 90 min. In the fourth, a 90-kg batch of milled maize was extracted for 90 min at 50 °C, the corn was separated from the extract using the decanter centrifuge and re-extracted with fresh aqueous ethanol at ambient temperature. The ethanol concentrations varied with the series and are described hereafter.

For batch 1 of the first series, the milled dent maize was added into 550 kg of a 60% ethanol solution in a jacketed, pressure tight, 1100-l tank (Lee Industries, Inc, Phillipsburg, PA). The slurry was agitated by propeller blades at 200 rpm and circulated out of the tank through a centrifugal pump (Fristram, model FP702, Middletown, WI) and back into the tank to increase the disruption

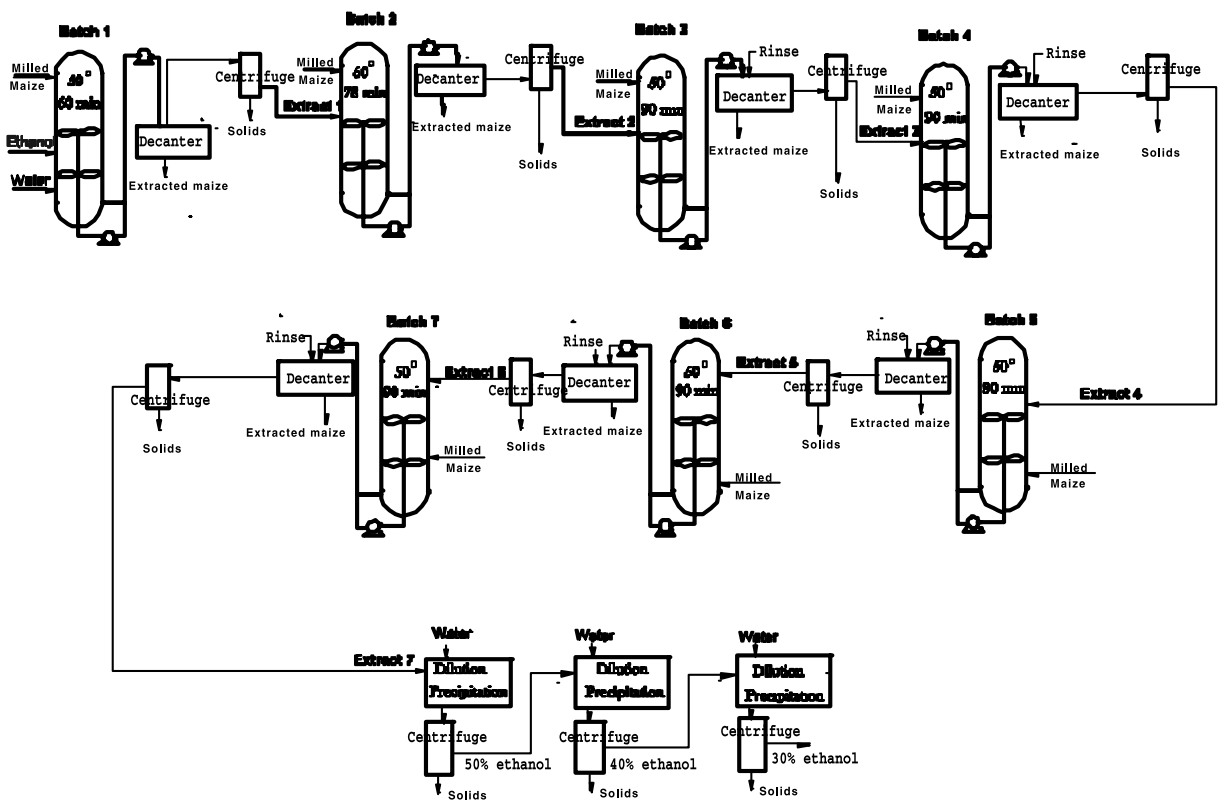

Fig. 1. Schematic diagram showing the experimental conditions for the seven-batch series of extractions.

of the endosperm structure. The temperature of the suspension was raised to 50 °C in a few minutes. The temperature was maintained for 1 h and then cooled to ambient temperature. This mixture was immediately pumped at 550 kg/h into a Super-D-Canter centrifuge (Sharples, model P-660, Warminster, PA) running at 6000 rpm ($3100 \times g$). The moist extracted maize (solid particles) and the extract (liquid) were collected at separate outlets.

The 507 kg of extract from batch 1 was used to extract a second 68 kg of milled maize, and subsequently processed in the same manner as batch 1. The 459 kg extracted from batch 2 was used in batch 3, which produced 396 kg. This was used to extract batch 4 to produce a final 344 kg of extract. For this series the bowl centrifuge (shown in Fig. 1 after the decanter centrifuge) was used.

The second series was similar to the first except that it started with 727 kg of 70% ethanol and each batch extracted 88 kg of milled maize. To reduce decanter clogging due to the buildup of fines, the extract decantate was pumped through a bowl centrifuge at the end of each batch. During the decanting of batch 3 and succeeding batches, a stream of 70% ethanol was pumped into the decanter centrifuge through a separate inlet and led to a central section where the ethanol solution rinsed the solids layer. The rinse rate was set to be sufficient to displace the decantate from the (approx. 55%) solids stream being pushed out of the decanter centrifuge. The rinse rate was usually one-third to one-fourth of the rate of the extraction vessel feed to the centrifuge. The extraction time was increased from 60 min for batch 1 to 78 min for batch 2 and 90 min thereafter.

The third series was similar to the first series except 90-kg batches were used and the series began with 727 kg of 70% ethanol. The batches were passed through a decanter centrifuge after extraction and batch 3 was rinsed during decanting with 15 kg of 70% ethanol.

In the fourth series, the milled maize was initially extracted with 409 kg of 70% ethanol. The extracted maize was then separated from the extract by decanter centrifugation and re-extracted without further drying, at ambient temperature (23 °C), with 409 kg of 70% ethanol. The re-extracted maize was separated from the extract with a decanter centrifuge and the extract centrifuged, diluted with water and re-centrifuged, as mentioned in the following section, at concentrations of 70, 50, 40 and 30% ethanol.

2.4. Fine solids separation

After the first series of extractions, the extract was stored indoors, at ambient conditions for a month after the last decanter centrifugation and pumped into a 10.5-cm tubular-bowl centrifuge (Sharples, division of Alfa Laval, model M-312-H-16, Warminster, PA) rotated at 15,000 rpm. The centrifuge generated $13{,}200 \times g$. Samples of the liquid centrifugate were collected for analysis and the rest of the centrifugate diluted to 50% ethanol, held overnight, and centrifuged until visibly clear of turbidity. The diluted extract separated by centrifugation was diluted to 40% ethanol, held overnight, and re-centrifuged until clear. Centrifugation in both cases required several days to complete.

The decantate fluids in the second, seven-batch series were centrifuged after each batch and the centrifugate reused to extract the next batch of maize. After batch 7, the decantate was centrifuged as before to remove fines and this centrifugate diluted to 50, 40 and 30% ethanol, with centrifuging after each dilution. The solid collected on the bowl was scraped out by hand, lyophilized, weighed and analyzed for protein, lipid and solids content.

The batch 3 decantate of the third series was collected in two parts and each part separately (bowl) centrifuged, diluted and re-centrifuged similar to the two prior series.

3. Results

Analysis of the samples from the first series of extractions indicates that the yields were not as high as the previous extractions carried out with 70% ethanol solution (Dickey et al., 2001). Therefore, the ethanol content of the initial extracting solution was increased to 70% for the later series.

Table 1
Decantate composition of a series of 1-h batch extractions with decantate reuse

Maize[c] extracted (kg)[a]	Decantate[a,b]				Time (h)[d]
	Mass (kg)	Solid (wt.%)	Protein (wt.%)	Lipid (wt.%)	
67.5	506.8	1.3	0.31	0.7	4
135.5	459.5	1.6	0.67	1.1	7.5
202.8	396.4	2.3	1.0	0.9	198
270.5	343.6	3.2	1.2	1.2	220

[a] Cumulative.
[b] Listed values include fines.
[c] Maize composition: solids 88%, protein 6.6%, lipids 7.2%.
[d] Period from the beginning of first extraction to sampling.

An inadvertent overrun on the time of batch 2 of the seven-batch series produced an increase in the extraction yield so the time was increased from 60 to 90 min for batch 3, and all subsequent batches. As indicated in Table 1, a rinse stream was used with the decanter centrifugation for the 90-min batches. During decanter centrifugation of the later batches of the four-batch series, the decanter plugged several times. We attributed this to the build up of fines in the extract. To prevent this plugging, the decanted extract was pumped through the bowl centrifuge to remove fines at the end of every batch in the seven-batch series.

The protein yields for the first series dropped slightly for each of the first three batches and markedly for the fourth batch. The fourth batch of the second series also had a lower fraction of the protein in the maize (fed to the fourth batch) transferred to the centrifugate as shown in Table 2 (column 6) with similarly low fractions for subsequent batches. The protein in the centrifuged (fines) solids increased to a peak of 4 kg for the fifth batch and decreased for the two subsequent batches. No starch was present in the fines for the first two (unrinsed) batches, 10% for the third (highest rinse rate) batch, and 2.6% for the last batch.

Rinsing the decanter centrifuge, as shown during the later five batches of the seven-batch series, increased the starch content of the fine solids exiting from the centrifuge suspended in the liquid stream. The correlation of starch content with rinse rate suggested that rinsing prevented thorough collection of the larger starch granules and corn particles in the decanter.

An extract of 378 kg containing 0.26% protein was recovered by decanter centrifugation of the initial extraction mixture. The extracted milled maize weighed 66.5 kg and contained 57% solids (43% evaporable ethanol and water) and 2–3.5% protein. After ambient re-extraction, the separated liquid contained 0.01% protein by measurement of samples of the liquid decanter output and 0.03% protein by calculation based on measurement of the weights and protein content of solids separated by the more accurate dilution and higher speed (bowl) centrifugation. The calculated protein of 0.03%, is 140 g, or approximately twice the estimated mass of protein in the 28.6 kg of liquid still on the extracted maize solid after decanter centrifugation based on it having the same composition as the extract.

4. Discussion

4.1. Protein extraction

Continuous, countercurrent extraction cannot be exactly replicated using any combination of batch extractions, but some limiting features of countercurrent extraction can be determined from a series of batch extractions. The fraction of the corn protein extracted and recovered in the extract listed in column 6 of Table 2 show that the protein extracted for each batch declined with

Table 2
Protein transfer in seven-batch extraction/decantation/centrifugation process

Batch[a]	Extracting liquid (kg) (% protein)	Decanted solids (kg) (% protein)	Decantate (kg) (% protein)		Fraction of corn protein[a] in extract	Extract time (min)	Decanter rinse (kg)	Time between extract decanting and centrifuging (days)
			Fine solids[b]	Extract[b]				
1	727, 0	130, 3.3	0.39, 0.06	656, 0.25	0.28	60	0	2
2	656, 1.6	136, 4.2	0.88, 0.23	577, 0.54	0.26	78	0	1
3	577, 3.1	135, 3.9	1.3, 0.31	552, 0.82	0.22	90	58	1
4	552, 4.4	142, 5.9	1.5, 0.63	493, 1.03	0.12	90	22	1
5	493, 5.1	142, 4.7	4.0, 1.52	446, 1.19	0.034	90	53	3, 4
6	446, 5.3	145, 6.6	3.3, 1.55	397, 1.44	0.070	90	48	5
7	397, 5.7	142, 6.2	2.6, 1.20	339, 1.60	0.00	90	41	2, 3

[a] 5.8 kg of corn (6.6% protein) was extracted for each batch.
[b] Separated by bowl centrifugation.

increasing batch number. This suggests that zein has limited solubility in the ethanol solution and further extraction is not possible in the extracting solution. When the maximum zein concentration in the extract is C_{sat}, and the concentration of zein at time t is $C_{(t)}$, a model where solute inhibits extraction in proportion to the difference, $C_{sat} - C_{(t)}$, will provide extraction rate, $dC_{(t)}/dt = k[C_{sat} - C_{(t)}]$. For continuous extraction unconstrained by extractable substrate availability with $C_{(0)} = 0$, integration gives $C_{(t)} = C_{sat}(1 - e^{-(kt)})$. In the series of batch extractions represented in Table 2 (column 5), zein concentration increased with batch number as the extract is exposed to more corn. The corn exposure per mass of extract is a measure of the (asymptotic) approach to a final extract composition and the sum of the batch solid/active liquid mass ratios (S/L) for the batch series is analogous to kt in the integrated continuous extraction with inhibition model. When the real situation is simplified by assuming that the maize was extracted under steady conditions, S equates to the product of (5.8 kg/90 min) and time of liquid exposure to extractable zein and L equates to the dwindling (727 → 400 kg) liquid mass. The ratio $S/L = 1$ corresponds to $kt = 1$ or $t = 1/k$, and from the data in Table 2, k equals approximately 7–8 h^{-1}. In Fig. 2, values of the solid to liquid mass ratio, the cumulative sum of the ratios of corn extracted divided by the extract (centrifugate) mass for that batch are plotted against the measured protein and lipid concentrations of the extracts for each batch. The batch extract protein values were fitted to the inhibiting solute continuous equation with cumulative values of (S/L) and $C_{sat} = 2.3$. The C_{sat} values indicate that zein concentrations above 2.3% cannot be obtained from a series of batch extractions, with cooling prior to separation of the extracted maize.

A 2.3 % protein concentration is well below the 5% concentration obtained by laboratory extractions of defatted flaked whole maize. Hojilla-Evangelista et al. (1992) made extractions by

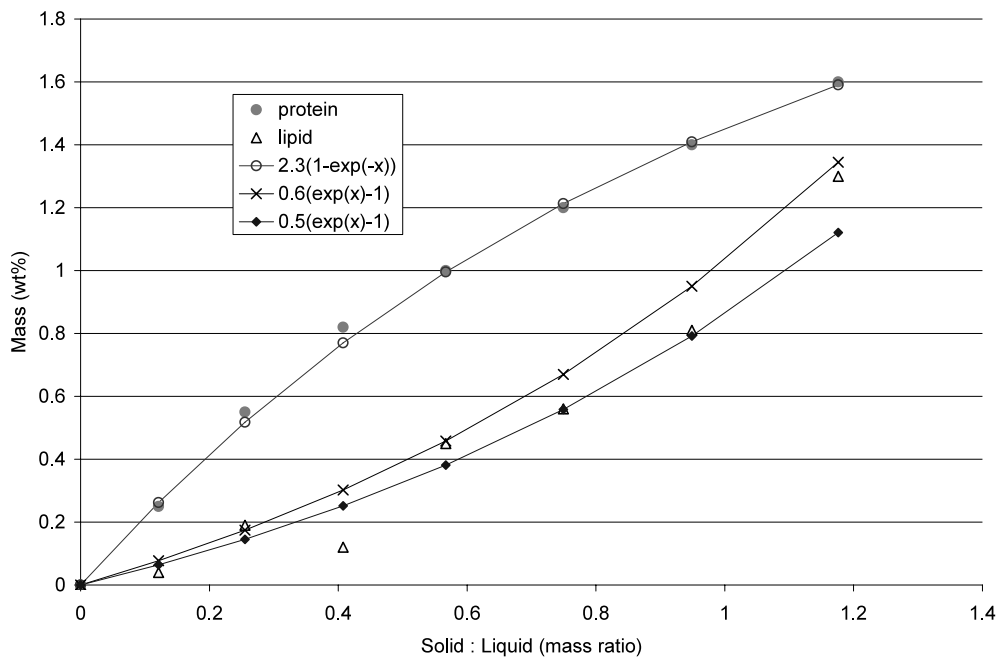

Fig. 2. Composition of centrifuged milled maize batch extract, with 70% ethanol. Protein and lipid curves are measured values, the upper connected curve is the form expected for extraction of an inhibiting protein, as discussed in Section 4.1. Protein extraction, the leading coefficient was obtained by fitting the measured protein data points. The two lower curves are fits to a simple exponential form that bound the lipid data points.

Table 3
Decantate composition from three 90-kg extraction batches

Maize[d] extracted (kg)[a]	Decantate[a,b]				Time (h)[c]
	Mass (kg)	Solid (wt.%)	Protein (wt.%)	Lipid (wt.%)	
88.2	676.4	0.69	0.35	0.20	4
174.9	615.7	1.43	0.61	0.49	30
263.5	582.8	1.93	0.79	0.56	76

[a] Cumulative.
[b] Including fines.
[c] From the beginning of first extraction to sampling.
[d] Maize composition: solids 87%, protein 7.6%, lipids 2.5%.

initially mixing 45% ethanol–55% 0.1 M NaOH (v/v) at a ratio of 15 ml/g of dry corn. The corn/liquid mixture was ground in a Waring blender for 1.5 min, soaked for 2 h, more liquid (13.5 ml/g of dry corn) was added, the mixture ground for another 30 s and the mixture was shaken in centrifuge bottles for 2 h at 55 °C. Finally, the bottles were centrifuged and the supernatant analyzed for protein. Although not explicitly stated, it is evident that the extract was not cooled before centrifugation.

Shukla et al. (2000) reported obtaining 1.6 (w/v)% zein concentration after six laboratory batch extractions of fresh milled maize by reusing extracts of preceding batches. A plot of zein concentration versus batch number indicated an increasing zein concentration with increasing batch. Each extraction was carried out for 30 min, with a 70% ethanol to solids ratio of 8:1, at 50 °C. These results are consistent with our larger scale results.

The 50% ethanol extraction in a screw conveyor of dried, defatted cracked maize had earlier been credited with the production of a solution containing approximately 2.8 (w/v)% zein (Chen, 1987). The L/S mass ratio of this example (I) was 0.75. Immediate centrifugation of corn gluten/extract mixture at its extraction temperature was also described in other zein extraction descriptions (Morris and Wilson, 1959; Carter and Reck, 1970; Takahashi and Yanai, 1996).

In contrast, the zein-like protein (gliadins) in wheat is soluble in 70% ethanol at low temperature (Rayas and Ng, 1997) and the wheat can be separated from the extract at ambient temperature and subsequently heated to precipitate the protein from solution. The extract zein limit shown in the seven-batch series is not due to insufficient extractable zein availability. The extracted zein should be at least as large as carefully extracted zein, which forms $16 \times 4.6 \times 4.6$ nm prisms in 70% ethanol (Matsushima et al., 1997). These species will be susceptible to aggregation and separation in the decanter or centrifuge after cooling to ambient. The different extract zein concentrations may be due to differences between the zein in the milled corn and the steeped zein in corn gluten. Composition and yield differences between the zein extracts of maize and corn gluten have previously been noted (Neumann et al., 1984; Wolf and Lawton, 1997; Parris et al., 1998) and have been explained as the result of sulfhydryl bond reduction in the corn gluten and removal of β- and γ-zeins. The zein derived from gluten has been found to be more soluble.

The increased protein contents of the decanted solids and fine solids for the later batches, shown in Table 2 (columns 3 and 4), are consistent with this interpretation. However, the increase in fine solids protein content may also be related to the increased time between decanting and centrifugation listed in Table 3 (column 6).

4.2. Lipid extraction

The lipid concentrations shown in Fig. 2 generally increased with the solid/liquid mass ratio with the difference between protein and lipid concen-

tration decreasing with increasing ratio. The measured values except for the one for batch 3 are bounded by the two exponential curves shown in Fig. 2. These curves have no theoretical significance, except to indicate that, unlike the protein, the lipid mass extracted increases with the solid/liquid mass ratio and is thus not inhibited and may be auto-catalytic over the range studied. Auto-catalytic in this case means increased lipid (or other solute) exposure (higher mass ratio) resulting in higher solute concentration enhances the solubility of lipid. The lipid results are much less accurate than those for the protein because of possible sampling errors. The lipid in the extracts was marginally stable, sometimes forming a separate phase during centrifugation. Previous results, where only a few batches had been run in series, had suggested that a longer series might produce a product with a high protein/lipid mass ratio. However, the seven-batch series contradicts that prediction. In fact, the major solid precipitated by dilution of the extract had a composition of fairly narrow range, and was independent of batch number or extract solute content. In light of a recent publication (Forato et al., 2000), the lipid in the major solid fraction precipitated is likely to be free fatty acid (FFA) closely associated with the zein. Their finding that protein bodies contain approximately 15% FFA, makes it more than possible that the FFA originally in the protein bodies resists centrifugation from 60 and 70% ethanol extracts until the extract is diluted to precipitate zein. The two-step extract dilution method removed triglyceride lipids from the extract in the first dilution, leaving a lower lipid content in the once diluted and centrifuged extract (Dickey et al., 2001).

Using the 15% FFA measurement, and the determination (Forato et al., 2000) that 63% of the FFA is linoleic acid (MW = 280), there are approximately 10 linoleic acid molecules per α-zein molecule (MW = 22,000) or approximately one linoleic molecule per helix. The zein SAXS measurements (Matsushima et al., 1997) indicated the short axis of the molecule is 1.4 nm and the length of a linoleic or oleic acid molecule is a zigzag 2.5 nm, matching the zein (helix) length. Thus linoleic or oleic acid molecules can fit between or within the α-zein helices. This arrangement is unlike the bi-layer model of commercial zein-oleic films proposed, based on X-ray measurements (Lai et al., 1999). Zein extracted and precipitated from the diluted extract, from which the triglycerides have been removed, should consist of a mix of FFA and protein more homogeneous than obtained using commercial purified zein mixed with fatty acids obtained independently.

Less lipids were apparently extracted in the third series (Table 3, column 5), than the first series (Table 1, column 5). The difference resulted from a difference in the maize composition. The lipid ratio of extractables (LRE), lipid/(lipid + zein), is 0.4 for both maize and the decantate samples in the third series. The LRE was 0.7 for the maize and for the first two batches of the first series, but decreased to 0.5 for the last two batches. This indicates that the decantate LRE is that of the maize when the lipid content does not exceed the saturation content of approximately 1.2%. In the second series, the LRE of the decantate was greater than the extract for the first batches, but converged to within a few percent for the last batches. The value decreased for the last batch and indicated that a higher protein fraction product will result from a series with a greater number of batches, or at higher solid/liquid mass ratios in a countercurrent extraction.

4.3. Rinsing extracted maize

The protein recoverable from the extracted maize by ambient rinsing is equal to the protein in the extract liquid left on the extracted maize and a similar, small, amount possibly due to newly extracted protein. The protein recoverable by rinsing, thus would increase as the protein concentration in the liquid increases with extract reuse. The mass ratio of liquid left on the extracted maize to liquid extract can be arbitrarily high at the beginning of a series of extractions, however, it will increase with increasing batch number with equal maize mass per batch due to liquid lost with the extracted maize. The loss can be overcome by rinsing the extracted maize. Protein recovery by rinsing will be less thorough as the solute concen-

tration increases, when the extracted protein aggregates as a result of cooling to ambient. Imperfect displacement of the extract on the extracted maize will inevitably occur, that is, some rinse liquid will bypass the extract on the corn and mix with the displaced extract. The decantate with which the rinse product liquid will be subsequently combined will be diluted by the combination. Overall, rinsing does not appear to be cost-effective in recovering solute protein associated with the extracted maize considering the low extract concentrations obtained in the longer series.

5. Conclusions

Ethanol solutions of native zein, prepared by reuse of extract were limited to less than 2%. The initially extracted zein precipitates during cooling and attaches to the extracted maize during decanter centrifugation or separates on its own in a subsequent centrifugation without water dilution. The solid recovered by centrifugation, before dilution, from the later batches of a series appears to be an inexpensive route to a zein product, possibly better than the one based on dilution.

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